IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Sackstein		
SERIAL NUMBER:	10/042,421	EXAMINER:	Phillip Gambel
FILING DATE:	October 18, 2001	ART UNIT:	1644
TITLE:	HEMATOPOIETIC CELL E-SELECTIN/ L-SELECTIN LIGAND POLYPEPTIDES AND METHODS OF USE THEREOF		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. § 1.132

I, Robert Sackstein, declare and state:

1. I am the sole inventor of the subject matter described and claimed in United States Patent Application Serial No. 10/042,421, filed October 18, 2001, entitled "Hematopoietic Cell E-Selectin/ L-Selectin Ligand Polypeptides and Methods of Use Thereof".
2. I have reviewed the Office Action dated August 20, 2009 and have reviewed the references cited therein. I make this declaration to rebut the Examiner's rejection, with which I do not agree.
3. I understand that the claim of the above-captioned application are directed to purified preparations of CD44 glycoproteins that comprise sialylated, fucosylated glycans and are ligands for E-selectin and/or L-selectin.
4. I understand the reference Lasky (U.S. Patent No. 5,652,343) is relied upon in a rejection under 35 U.S.C. § 103(a), which regards the issue of obviousness. Particularly, Lasky is being relied upon for its teaching of purification of L-selectin ligand using "L-selectin-immunoglobulin chimera" (hereinafter "L-selectin-Ig chimera"), which the examiner considers a "key starting material" that may be used to isolate the claimed CD44 glycoproteins. The discussion and evidence presented in

this declaration make clear that L-selectin Ig-chimera does not bind the CD44 glycoproteins of the present claims.

5. First, Lasky clearly uses the L-selectin-Ig chimera to obtain *MECA-79 reactive antigen that is sulfated*[1/], and further, that "the glycoprotein ligands of the present invention comprise fucose, sialic acid and an anionic component, preferably sulfate esters as O-linked carbohydrate components, and it is believed that fucose, like sialic acid, and sulfate are required for full ligand activity."[2/] Thus, Lasky cannot be combined with Sackstein 1997[3/], which specifically discloses an L-selectin ligand that does *not contain MECA-79 antibody-specific epitopes and is not dependent on sulfation for ligand activity.* Indeed, L-selectin-Ig chimera binding to L-selectin ligand(s) specifically requires sulfation of the target ligands (i.e., L-selectin-Ig chimera is biased toward detection of sulfated L-selectin ligands (see Point (10), below).

6. Further, **L-selectin-Ig chimera does not recognize the CD44 glycoproteins of the present claims.** While L-selectin-Ig chimera has been used to immunoprecipitate L-selectin ligands from lymph node high endothelial venules (all of which are reactive with mAb MECA 79 and are sulfate-dependent ligands, see Point (10) below), this reagent does not recognize the CD44 glycoproteins of the present claims. As shown in the attached primary lab data and paperwork, I, with the help of members of my lab, made several attempts to purify the glycoprotein described in Sackstein 1997 (my own work) with L-selectin-Ig chimera. My lab had generated L-selectin-Ig chimera, and I also obtained L-selectin-Ig chimera from various other sources (including a commercial source [R&D Systems], from Genetics Institute (a biotechnology company), and from the laboratory of Dr. Ajit Varki of the University of California, San Diego). I also obtained purified L-selectin commercially (from R&D Systems) and also purified L-selectin myself from human lymphocytes. I exhaustively tried to use these reagents to identify the novel L-selectin ligand (*i.e.*, HCELL) expressed on KG1a cells. The L-

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1/ See Lasky at column 4, lines 46 - 65 (Summary of the Invention)(stating that "L-selectin-Ig chimera to precipitate inorganic sulfate-labeled material" which was "abolished by treatment of the sulfate-labeled proteins with sialidase" and, further, that the "monoclonal antibody, termed MECA-79,...precipitated both components.").

2/ See Lasky at column 13, lines 33-37.

3/ Sackstein *et al.*, Blood. 1997 Apr 15;89(8):2773-81.

selectin-Ig chimera were complexed to Protein A-agarose beads, packed in columns, and lysates of KG1a cells were passed over such columns in the presence of calcium. We also directly complexed purified L-selectin to agarose beads and passed lysates of KG1a cells over these L-selectin-bearing beads in the presence of calcium. No L-selectin ligands were purified. Moreover, in every case, using either L-selectin-Ig chimera or purified L-selectin, I was unable to stain KG1a cells by flow cytometry (see accompanying Figure) and was unable to immunoprecipitate any molecules from lysates of KG1a cells using L-selectin-Ig chimera in suspension.

7. The attached Figure shows the results of an experiment conducted June 13, 1996. In that Figure, data are shown using L-selectin-Ig chimera (called "L-selectin chimera", produced by us) and P-selectin-Ig chimera (called "P-selectin chimera", provided by the Genetics Institute) to probe by flow cytometry for expression of L-selectin ligand(s) and of P-selectin ligand(s), respectively, on KG1a cells. These probes were labeled with fluorochrome (FITC), then incubated with KG1a cells in the presence of calcium. Note that L-selectin-Ig chimera does not stain the KG1a cells (the fluorescence signal on the X-axis ("FL-1") is at the origin (0-10), i.e., it fails to detect any L-selectin ligand(s)). On the other hand, P-selectin-Ig stains the cells (fluorescence signal is at 100-1000 intensity, showing that there is a P-selectin ligand detectable by flow cytometry). Thus, although there was clear operational evidence of an L-selectin ligand using a functional shear-based assay (the Stamper-Woodruff assay), this same L-selectin ligand (i.e., HCELL) is UNDETECTABLE using L-selectin-Ig chimera.

8. Even had I – or others – obtained evidence that L-selectin-Ig chimera could recognize the novel L-selectin ligand now known as HCELL, use of L-selectin-Ig chimera alone would not be sufficient to isolate HCELL, because hematopoietic cells express multiple L-selectin and E-selectin ligands, including the pan-selectin ligand known as P-selectin Glycoprotein Ligand- 1 (PSGL-1). In particular, Spertini *et al.* published in 1996 (Journal of Cell Biology 135:523-531) that L-selectin-Ig chimera can recognize PSGL-1 expressed on hematopoietic cells. Importantly, these investigators showed that L-selectin-Ig chimera staining of KG-1 cells (the parental line to KG1a cells) was completely abrogated by treatment of KG-1 cells with O-sialoglycoprotein endopeptidase (also known as "O-sialoglycoprotease" or "OSGE") (see Figure 4) and

was eliminated by treatment of the cells with chlorate (to inhibit sulfation) or by desulfation using sulfatase (see Figure 2); these investigators went on to show that L-selectin-Ig chimera binding to the sulfation-dependent and OSGE-sensitive L-selectin ligand of KG-1 cells was blocked by mAb directed to PSGL-1, *i.e.*, the target molecule was PSGL-1 (see Figures 6 and 7 of text).

9. In contrast, our work definitively showed that the L-selectin ligand activity of HCELL was not affected by OSGE treatment (see Table 2 in Oxley and Sackstein, Blood 84:3299-3206; 1994) and that the novel L-selectin ligand (*i.e.*, HCELL) expressed on KG1a cells displayed sulfation-independent ligand activity (see Sackstein et al, Blood 89:2773-2781, 1997).

10. Notably, Lasky, Rosen and coworkers reported that L-selectin-Ig chimera binding to L-selectin ligands is critically dependent on ligand sulfation, i.e., binding does not occur if the L-selectin ligand is *not sulfated* (see Imai et al, Nature 361: 555-557, 1991; see Hemmerich et al, Journal of Experimental Medicine 180:2219-2226, 1994; see Yoshida et al, European Journal of Biochemistry 222:703-709, 1994). These findings are consistent with that of Spertini et al. (see Point (8) above) who showed that L-selectin-Ig chimera recognition of PSGL-1 is sulfation-dependent. Thus, HCELL, a sulfation-independent L-selectin ligand, is not identified by L-selectin-Ig chimera, i.e., HCELL lacks the critical sulfate-dependent "anionic component" described in Lasky that is necessary for L-selectin-Ig chimera recognition of target ligand(s).

11. In addition to the fact that the HCELL binding determinant for L-selectin lacks sulfation, L-selectin-Ig chimera (and purified L-selectin, for that matter) repeatedly failed to identify the novel L-selectin ligand (*i.e.*, HCELL) because this ligand requires a critical hemodynamic shear to engage L-selectin. The identification of the HCELL molecule was highly elusive because the binding of L-selectin is crucially shear-dependent, in other words, L-selectin binding to HCELL does not occur under static conditions. Thus, previous attempts at "ligand blotting" using L-selectin-Ig chimera and purified L-selectin to probe western blots of KG1a cell lysates failed to identify HCELL because these techniques lack critical shear (i.e., ligand blotting using L-

selectin-Ig chimera as probe failed for this reason, in addition to the reason that L-selectin-Ig chimera only recognizes *sulfated L-selectin ligands*).

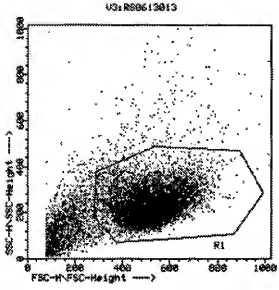
12. I thus had to specifically create a totally new technology (the "blot rolling assay") to identify the novel L-selectin ligand (*i.e.*, HCELL) expressed on KG1a cells. This technology was first described in Dimitroff *et al.*, Proceedings of the National Academy of Sciences 97:13841-13846, 2000. The major advantage of this method is that it allows for the rapid and reproducible assessment under appropriate functional shear stress of selectin ligands within a complex mixture of molecules without the need for prior isolation or enrichment of the constituent parts beyond an electrophoretic step and subsequent western blotting. The resultant blotting membrane is rendered semi-transparent by pre-incubation in glycerol-containing buffers, and cells or particles (*e.g.*, microbeads) bearing adhesion molecules of interest (*e.g.*, L-selectin-bearing lymphocytes) are introduced under appropriate physiological flow conditions and observed by video microscopy for interaction with pertinent immobilized ligands. Tethering and rolling interactions are observed directly on the target ligand band(s). The band(s) supporting such adhesive interactions can then be excised and subjected to mass spectrometry (or protein sequencing) for identification. This inventive step was required to identify the HCELL molecule.

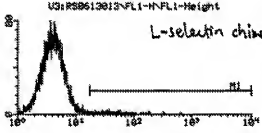
13. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Robert Sackstein

1/15/10
Date

Figure





V3:RS8613013\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for V3:RS8613013
Selected Preferences: Arithmetic/Linear

Parameter FL1-H	FL1-Height	Gate G1= R1				
M	Left,Right	Events	%	Peak	PkChl	Mean
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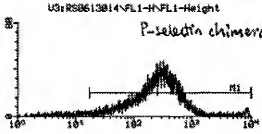
1	17.62, 9910	141	1.79	4	37.52	58.14



V3:RS8613014\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for V3:RS8613014
Selected Preferences: Arithmetic/Linear

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1	17.62, 9910	7589	95.79	47	324.00	498.91	